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Protein digestibility evaluations of meat and fish substrates using laboratory, avian, and ileally cannulated dog assays[1]

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ABSTRACT: Meat and fish serve as important protein sources in the companion animal diet; however, limited protein digestibility data are available for assessing protein digestibility differences among good-quality protein sources. Beef loin, pork loin, chicken breast, pollock fillet, and salmon fillet were evaluated for composition, protein digestibility, and AA bioavailability using the immobilized digestive enzyme assay, cecectomized rooster assay, and ileally cannulated dog assay. Pollock contained the greatest amount of CP, total essential AA (TEAA), and total nonessential AA (TNEAA; DM basis; 96.9, 38.6, and 50.3%, respectively). Salmon contained the next greatest amounts (92.8, 36.4, and 44.6%), followed by chicken (90.3, 36.1, 43.2%). Beef had the least CP content (82.7%), but had slightly greater TEAA and TNEAA concentrations (33.9, 42.0%) compared with pork (86.2, 33.6, 41.3%). Immobilized digestive enzyme assay values were greatest for pollock fillet (0.71) and least for chicken breast (0.52). Beef loin, pork loin, and salmon fillet were similar (0.63, 0.62, and 0.64, respectively). Standardized TEAA and TNEAA digestibility coefficients, evaluated using the cecectomized rooster assay, were greatest ($P < 0.05$) for pollock fillet (90.4 and 89.8%, respectively) and least ($P < 0.05$) for chicken breast (86.6 and 85.9%, respectively) and salmon fillet (87.8 and 86.4%, respectively). Dogs assigned to a 5 × 5 Latin square design were fed 5 diets, with each test substrate as the major protein source. No significant differences ($P > 0.05$) were found in ileal digestibility of protein. Values ranged from 88.9% for chicken to 90.5% for pork loin and pollock fillet. Ileal TEAA and TNEAA coefficients were not different among test substrates, with values between 91.7 and 92.7%, and 88.8 and 90.4%, respectively. Total tract CP apparent digestibility values ranged from 94.4 to 94.8%, with no differences noted among treatments. Despite marked differences in composition and predicted and standardized digestibility values, when the protein sources were added to diets at a concentration of approximately 30% (25% of total energy intake), no differences in test protein substrates were noted in either ileal or total tract nutrient digestibility.

Key words: amino acid bioavailability, animal protein, canine, digestibility, fish

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INTRODUCTION

Beef, pork, chicken, and fish serve as important essential and nonessential AA sources in the companion animal diet. Because of the humanization of pets and concern for better pet health, owners are demanding pet diets that include good-quality ingredients, leading to a 30% increase in good-quality pet food sales between 2004 to 2009 (Tait, 2004). This trend is expected to continue. Owners prefer that diets contain meat instead of meat by-products as a sign of good quality and as a dietary ingredient whose background they understand (McBride, 2003).

It is important to understand the compositional and digestibility differences that exist among animal and marine proteins. Information is available on meat and fish composition (Novakofski et al., 1989; Browning et al., 1990; Suvanich et al., 1998; Bechtel, 2003; Lonergan et al., 2003; Husak et al., 2008); however, data are limited on nutrient digestibility as affected by protein source. Fish is thought to be a superior source of protein, based on nutrient composition and simple protein quality assays (biological value and chemical score) re-

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ported in the USDA Nutrient Database (Sheeska and Murkin, 2002). However, a comparative study of compositional and digestibility differences comparing marine to mammalian and avian protein sources has yet to be conducted.

The objective of this study was to evaluate composition and protein digestibility differences among 3 meats (beef loin, pork loin, and chicken breast) and 2 fish (Alaskan pollock fillet and pink salmon fillet). This was accomplished by conducting detailed chemical compositional analyses and by evaluating all substrates by using the immobilized digestive enzyme (**IDEA**) and cecectomized rooster assays. In addition, each test substrate was added to diets fed to ileally cannulated dogs for the purpose of obtaining ileal and total tract nutrient digestibility coefficients.

MATERIALS AND METHODS

Animal care procedures were approved by the University of Illinois Institutional Animal Care and Use Committee.

Substrates

Beef loin, pork loin, chicken breast, salmon fillet, and pollock fillet were evaluated in this study. Beef loin, pork loin, and chicken breast were provided by the University of Illinois Meat Science Laboratory, Urbana. Good-quality cuts with a relatively small fat content were chosen. Substrates were frozen and shipped to the University of Alaska, Fairbanks, for further processing. Skinless Alaskan pollock and pink salmon fillets were prepared and frozen by a commercial fish processor in Kodiak, AK, using commercial processing equipment. No additives, such as salt or antioxidants, were used for meat or fish preservation.

All substrates were dried in an Enviro-Pak dryer (Shelf Pak Series, model CHU 150E, Enviro-Pak, Clackamas, OR) with a dehumidifying attachment to allow low-temperature drying. Substrates were dried for 6 h at 71°C. All thaw times, dry times, and dryer load sizes were kept consistent. Once dried, substrates were refrigerated and shipped to the University of Illinois, Urbana, and ground to a 2-mm particle size using a commercial bowl chopper for meat (model C40P, Talsa, St. Paul, MN).

Chemical Analyses

Meat and fish substrates were analyzed for DM, OM, and ash using AOAC (2006) methods (methods 934.01, 942.05). Crude protein was calculated from Leco total N values (AOAC, 2006; method 992.15). Total lipid content (acid-hydrolyzed fat) of the substrates was determined according to the methods of the American Association of Cereal Chemists (1983) and Budde (1952). Gross energy of the substrates was measured using an oxygen bomb calorimeter (model 1261, Parr Instruments, Moline, IL). Total dietary fiber was analyzed according to the method of Prosky et al. (1984). Biogenic amine concentrations were measured by HPLC according to methods described by Flickinger et al. (2003). Long-chain fatty acids were measured by gas chromatography according to the method of Lepage and Roy (1986). Amino acids (University of Missouri Experiment Station Chemical Laboratories, Columbia; AOAC, 2006; method 982.30E) and minerals (University of Missouri Experiment Station Chemical Laboratories; inductively coupled plasma-optical emission spectroscopy; AOAC, 2005; methods 985.01A, B, D) were evaluated. Collagen, expressed as a percentage of CP concentration, was calculated by multiplying hydroxyproline concentrations by a factor of 8, and then dividing by the CP percentage (AOAC, 2006; method 990.26). Compositional data are presented in Table 1. Compositional data were not analyzed using statistical methods because accuracy is ensured by adequate replication, with acceptance of mean values that are within 5% of each other.

IDEA

A Poultry Complete IDEA kit (Novus International Inc., St. Charles, MO; Schasteen et al., 2002) was used to evaluate meat and fish in vitro digestibility of CP and AA. Test substrates were ground to an average 1-mm particle size and solubilized in 50 mL of Poultry Complete Solubilization Solution (Novus International Inc.). The solution components were potassium dihydrogen phosphate, sodium azide, and EDTA. The solubilized sample was transferred to a digester tube containing proprietary lyophilized enzymes provided in the kit and was mixed for 2 h at 37°C. During this step, peptide bonds were hydrolyzed by enzymes, freeing α-AA. Once digested, 80 mg of orthophthaldialdehyde (**OPA**) solution and 0.2 mL of mercaptoethanol were added to each tube so that OPA might bind to the freed α-AA. Absorbance of OPA (340 nm) was measured, using a spectrophotometer, in an untreated sample, the digested sample, and an enzyme blank. An IDEA value was calculated by subtracting absorbencies of the initial and enzyme blank samples from the digested sample. This value was divided by the CP percentage. This value reflects the number of AA freed by enzyme hydrolysis. Amino acid digestibility values were calculated by multiplying the IDEA value by regression equations based on cecectomized rooster assay data (Schasteen et al., 2002). Because of the nature of the IDEA assay, only one data point is obtained. Statistical analysis is not possible with this assay.

Cecectomized Rooster Assay

A cecectomized rooster assay was conducted as described by Sibbald (1979) to evaluate standardized AA

Table 1. Chemical composition of meat and fish substrates (DM basis except for DM, %)

Item	Beef loin	Pork loin	Chicken breast	Pollock fillet	Salmon fillet
DM, %	95.3	95.1	92.6	95.6	92.7
OM, %	95.8	95.4	95.9	93.7	94.8
Ash, %	4.2	4.6	4.1	6.3	5.2
CP, %	82.7	86.2	90.3	96.9	92.8
Acid-hydrolyzed fat, %	16.4	15.4	11.1	4.5	7.6
Total dietary fiber, %	1.2	0.3	1.2	0.3	0.2
GE, kcal/g	6.0	5.9	5.8	5.3	5.6
AA, %					
Essential					
Histidine	2.95	3.12	3.14	1.95	2.15
Isoleucine	3.77	3.67	4.02	4.25	4.06
Leucine	6.53	6.32	6.78	7.59	6.82
Lysine	6.87	6.88	7.48	8.67	7.80
Methionine	2.10	2.11	2.31	2.87	2.62
Phenylalanine	3.24	3.14	3.34	3.62	3.46
Threonine	3.45	3.43	3.63	3.93	3.85
Tryptophan	0.93	0.93	1.10	0.96	1.05
Valine	4.05	3.96	4.31	4.75	4.62
Nonessential					
Alanine	4.62	4.52	4.91	5.55	5.12
Arginine	5.08	5.01	5.44	5.92	5.12
Aspartic acid	7.23	7.12	7.73	9.31	8.31
Cysteine	0.82	0.64	0.83	0.92	0.80
Glutamic acid	11.34	10.93	11.49	13.33	11.51
Glycine	3.70	3.74	3.76	4.48	4.02
Hydroxylysine	0.04	0.03	0.02	0.05	0.03
Hydroxyproline	0.39	0.41	0.25	0.23	0.16
Lanthionine	0.24	0.28	0.28	0.00	0.19
Ornithine	0.08	0.07	0.08	0.12	0.08
Proline	2.93	2.90	2.83	2.98	2.76
Serine	2.74	2.77	2.94	3.56	3.10
Taurine	0.09	0.17	0.05	0.60	0.43
Tyrosine	2.73	2.72	2.56	3.21	2.97
TEAA[1]	33.9	33.6	36.1	38.6	36.4
TNEAA[2]	42.0	41.3	43.2	50.3	44.6
TAA[3]	75.9	74.9	79.3	88.9	81.0
Total fatty acids, mg/g	196.77	197.40	127.37	47.97	79.44
Total SFA	57.60	47.99	31.60	7.43	15.06
Total MUFA	63.63	60.49	38.98	5.46	20.20
Total PUFA	11.46	24.78	13.15	30.02	33.06
Total BCFA[4]	2.09	0.13	0.32	0.03	0.30
Total n-3	0.89	1.37	0.89	28.18	30.92
Total n-6	10.25	23.55	11.46	1.85	1.88
Biogenic amine, μmol/g					
Tryptamine	0.00	0.00	0.00	0.00	0.04
Phenylethylamine	0.00	0.00	0.00	0.14	0.00
Putrescine	0.13	0.03	0.09	0.24	0.51
Cadaverine	0.09	0.00	0.00	0.00	0.00
Histamine	0.00	0.00	0.00	0.00	0.00
Tyramine	0.72	0.00	0.00	0.00	0.00
Spermidine	0.04	0.07	0.12	0.00	0.13
Spermine	0.25	0.69	0.92	0.01	0.16
Mineral					
Ca, %	0.03	0.03	0.02	0.06	0.06
P, %	0.77	0.89	0.88	0.96	1.04
Mg, %	0.09	0.11	0.11	0.15	0.12
Hg, mg/kg	<0.075[5]	<0.075[5]	<0.075[5]	<0.075[5]	<0.075[5]
Fe, mg/kg	94.00	35.00	21.00	45.00	35.00
Zn, mg/kg	137.00	82.00	24.00	20.00	17.00

[1]TEAA = total essential AA.
[2]TNEAA = total nonessential AA.
[3]TAA = total AA.
[4]BCFA = total branched-chain fatty acids.
[5]Below method detection limit.

digestibility of the 5 meat and fish substrates. Single Comb White Leghorn roosters (n = 20; approximately 50 wk of age) were used in this study. At age 25 wk, all roosters underwent a cecectomy under general anesthesia following the methods of Parsons (1985). Roosters were allowed to recover for 8 wk after surgery before being used in experiments. Roosters were individually housed in raised wire cages in an environmentally controlled room with a 16-h light:8-h dark cycle. Roosters had ad libitum access to food and water before beginning the experiment.

Roosters were fasted for 24 h before being dosed with the test substrates. Each rooster was crop-intubated and given 30 g of 1 test substrate (4 roosters per test substrate evaluated). After crop intubation, roosters were again fasted and all excreta were collected on a plastic tray under the cage for 48 h. Excreta were freeze-dried, weighed, and ground through a 0.25-mm screen. Amino acid concentrations were measured in each sample (University of Missouri Experiment Station Chemical Laboratories; AOAC, 2006; method 982.30E). Endogenous excretion of AA was measured using roosters that were fasted for 48 h. The latter values were used to calculate standardized AA digestibility values, using the method described by Sibbald (1979).

Statistics were conducted using ANOVA for a completely randomized design (SAS Inst. Inc., Cary, NC). Differences among treatments were determined using LSD values calculated from the pooled SEM. A *P*-value of <0.05 was accepted to denote statistical significance.

Animals and Diets

Five female ileally cannulated hound-mix dogs (5.6 ± 2.4 yr; 23 ± 1.3 kg) were used. Dogs had been surgically prepared with a T-shaped cannula according to the procedure of Walker et al. (1994). Dogs were housed in individual kennels (2.4 × 1.2 m) in a temperature-controlled room (22°C; 23% relative humidity) with a 16-h light:8-h dark cycle.

Five diets were formulated to contain approximately 30% protein and 20% fat. This nutrient composition is representative of a good-quality commercial dog diet. Each diet contained 1 test protein source (beef loin, pork loin, chicken breast, pink salmon fillet, or Alaskan pollock fillet) as the primary protein source. Poultry fat, brewers rice, ground corn, beet pulp, and vitamin and mineral supplements made up the remainder of the dry kibble diet. Diets were extruded at the Kansas State University Bioprocessing and Industrial Value-Added Program facility (Manhattan) under the supervision of Pet Food and Ingredient Technology Inc. (Topeka, KS). Dogs were offered 150 g of the diet twice daily (0800 and 2000 h). Chromic oxide (0.2%) was added to the diet as a digestibility marker. Fresh water was offered ad libitum.

Sample Collection

A 5 × 5 Latin square design with 14-d periods was conducted. The first 10 d was an adaptation period, followed by 4 d of ileal and total fecal collection. Ileal effluent was collected 3 times/d every 4 h. Ileal collection times were adjusted by 1 h from the collection time of the previous day. For example, sample times on collection d 1 were 0800, 1200, and 1600 h; on d 2, samples were collected at 0900, 1300, and 1700 h; and so on. Ileal effluent was collected into a sterile sampling bag by attaching the bag to the cannula extension with a rubber band. Before bag attachment, cannula barrels were scraped clean using a spatula. During collections, dogs wore Bite-Not collars (Bite-Not Products, San Francisco, CA) to prevent the dog from removing the sample bag. Dogs were encouraged to move freely during collections. After collection, a cannula plug was placed in the barrel and the cannula site was cleaned with a dilute Betadine solution (1.0% solution, Purdue Frederick Company, Stamford, CT).

Although total tract nutrient digestibility was based on the concentration of chromic oxide recovered, total feces excreted during the collection phase of each period were collected from the pen floor, weighed, and frozen at −20°C until further analyses. All fecal samples during the collection period were subjected to a consistency score according to the following scale: 1 = hard, dry pellets, small hard mass; 2 = hard, formed, dry stool, remains firm and soft; 3 = soft, formed, moist stool, retains shape; 4 = soft, unformed stool, assumes shape of container; 5 = watery liquid that can be poured.

Sample Handling

Ileal samples were frozen at −20°C in their individual bags until further analyses. After all samples were collected, ileal effluent from each dog was composited by period and refrozen at −20°C. Ileal effluent was then lyophilized in a Dura-Dry MP microprocessor-controlled freeze-dryer (FTS Systems, Stone Ridge, NY). Once dry, ileal effluent was ground with a mortar and pestle to a 2-mm particle size. Fecal samples were dried at 55°C in a forced-air oven and ground in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) through a 2-mm screen. On d 11 of each period, fresh fecal samples were collected within 15 min of defecation. Aliquots for analysis of phenols, indoles, and biogenic amines were frozen at −20°C immediately after collection. One aliquot was collected and put in 5 mL of 2 *N* HCl for ammonia analysis. Additional aliquots were used for pH measurement and fresh fecal DM determination.

Chemical Analyses

Diet, ileal, and fecal samples were analyzed for the same constituents as the test substrates. In addition,

Cr concentrations of diet, digesta, and fecal samples were analyzed according to the method of Williams et al. (1962), using atomic absorption spectrophotometry (model 2380, Perkin-Elmer, Norwalk, CT). Ammonia concentrations were determined according to the method of Chaney and Marbach (1962). Phenol and indole concentrations were determined by gas chromatography according to the methods described by Flickinger et al. (2003). Biogenic amines concentrations were measured by HPLC according to the methods described by Flickinger et al. (2003).

Calculations

Dry matter recovery was calculated by dividing Cr intake (mg/d) by Cr concentrations in ileal effluent (mg of Cr/g of ileal effluent). Ileal nutrient flows were calculated by multiplying DM flow by the nutrient concentration in the ileal DM. Ileal nutrient digestibility values were calculated as nutrient intake (g/d) minus ileal nutrient flow (output, g/d); this value was then divided by nutrient intake (g/d). Similar calculations were performed on fecal samples to determine total tract nutrient digestibility values.

Data were analyzed using the MIXED procedure (SAS Inst. Inc.). The statistical model included period and dog as random effects, whereas treatment was a fixed effect. Digestibility calculations were analyzed using the type 3 tests of the MIXED procedure, whereas protein catabolites were analyzed using the REML estimation of the MIXED procedure because of the presence of 0 values in the data set. Means were separated using a protected least squares difference with a Tukey adjustment. A probability of $P < 0.05$ was accepted as statistically significant.

RESULTS

Substrate Composition

Meat and fish substrate compositional data are presented in Table 1. Substrate DM concentrations were similar among substrates. Fish fillets contained more ash than beef loin, pork loin, or chicken breast. Crude protein concentrations were greatest in pollock fillet and least in beef loin. Acid-hydrolyzed fat concentrations were the inverse, with beef loin having the greatest and pollock fillet the smallest fat content. Total dietary fiber, a measure of connective tissue concentration, was low for all substrates, with beef loin and chicken breast having the greatest concentrations. Beef loin had the greatest GE content and pollock fillet had the least.

Pollock fillet contained the greatest concentrations of each AA except for histidine and tryptophan. Beef loin and pork loin had similar concentrations of each AA. Total AA (**TAA**) concentrations were greatest in pollock fillet and least in pork loin, as were total essential AA (**TEAA**) and total nonessential AA (**TNEAA**) concentrations.

Beef loin and pork loin had the greatest SFA concentrations, whereas salmon fillet and pollock fillet had the least. Monounsaturated fatty acid concentrations were greatest in beef loin and least in pollock fillet. Beef loin contained the greatest concentration of branched-chain fatty acids, and pollock fillet had the least. Total PUFA concentrations were greatest in salmon fillet and least in beef loin.

Salmon fillet and pollock fillet contained the greatest concentrations of n-3 fatty acids. Concentrations were least in beef loin and chicken breast. Omega-6 fatty acid concentrations were greatest in pork loin and least in salmon and pollock fillets.

Spermine and spermidine were present in chicken breast, pork loin, beef loin, and salmon fillet. Putrescine was greatest in salmon fillet and least in pork loin. Tyramine and cadaverine were present only in beef loin. Tryptamine was present only in salmon fillet, and phenylethylamine was present only in pollock fillet. Histamine was not detected in any of the substrates.

Calcium concentrations were greatest in pollock and salmon fillets, whereas chicken breast contained the least Ca. Phosphorus concentrations were greatest in salmon fillet and pollock fillet and least in beef loin. Magnesium concentration was greatest in pollock fillet and least in beef loin. Iron concentration was greatest in beef loin compared with the other substrates, which were similar in Fe content. Zinc was greatest in beef loin and pork loin. Chicken breast, pollock fillet, and salmon fillet were least in Zn content. Mercury was not detected in the substrates. Beef loin contained the greatest collagen concentration (4.8%), followed by pork loin (3.8%), chicken breast (2.2%), salmon fillet (2.0%), and pollock fillet (1.3%; data not shown).

IDEA

The IDEA value was greatest for pollock fillet (0.71) and least for chicken breast (0.52; Table 2). All individual AA digestibility values were greatest for pollock fillet and least for chicken breast, as were TAA, TEAA, and TNEAA values.

Cecectomized Rooster AA Digestibility

Standardized AA digestibility coefficients are presented in Table 3. All individual AA digestibility values differed ($P < 0.05$) among substrates, except for lysine and cysteine. Pollock fillet TEAA digestibility (90.4%) was greatest ($P < 0.05$) compared with all other substrates. Beef loin, pork loin, and salmon fillet did not differ in TEAA digestibility (89.0, 89.0, and 87.8%, respectively). Chicken breast TEAA digestibility (86.6%) was less ($P < 0.05$) compared with other substrates, but was similar to that of salmon fillet. Total nonessential AA digestibility was greatest ($P < 0.05$) for pollock

Table 2. Immobilized digestive enzyme assay (IDEA) values and predicted AA digestibility values for meats and fish

	Substrate				
Item	Beef loin	Pork loin	Chicken breast	Pollock fillet	Salmon fillet
IDEA value	0.63	0.62	0.52	0.71	0.64
AA					
Essential					
Histidine	89.6	88.7	79.1	97.9	91.1
Isoleucine	93.4	92.9	86.3	96.6	94.1
Leucine	94.0	93.6	87.3	97.5	94.8
Lysine	92.0	91.6	83.0	93.9	92.7
Methionine	93.7	93.2	85.7	97.7	94.6
Phenylalanine	95.8	95.3	89.1	101.2	96.9
Threonine	90.4	89.9	82.7	94.6	91.3
Tryptophan	92.6	92.5	90.2	93.2	92.8
Valine	93.9	93.1	84.1	101.8	95.4
Nonessential					
Alanine	91.9	91.3	85.0	97.4	92.9
Arginine	92.5	92.1	87.7	96.3	93.2
Aspartic acid	81.7	80.7	68.8	92.0	83.6
Cysteine	79.1	78.1	64.3	87.6	80.9
Glutamic acid	92.3	91.6	83.2	99.5	93.6
Proline	91.3	90.7	84.2	97.0	92.4
Serine	89.9	89.1	80.8	97.1	91.2
Tyrosine	95.3	94.9	89.7	99.9	96.2
TEAA[1]	92.8	92.3	85.3	97.2	93.7
TNEAA[2]	89.3	88.6	80.5	95.9	90.5
TAA[3]	91.1	90.5	83.1	96.5	92.2

[1]TEAA = total essential AA.
[2]TNEAA = total nonessential AA.
[3]TAA = total AA.

fillet (89.8%). Beef loin (88.1%) and pork loin (87.9%) were not different in TNEAA digestibility. Chicken breast (85.9%) and salmon fillet (86.4%) had smaller ($P < 0.05$) TNEAA digestibility values than other substrates, but values did not differ between the 2 substrates. Total AA digestibility was greater ($P < 0.05$) for pollock fillet (90.4%) compared with all other substrates. Beef loin (89.1%) and pork loin (89.1%) were intermediate and were not different from each other. Chicken breast and salmon fillet had smaller ($P < 0.05$) TAA digestibility values compared with all other substrates (86.9 and 87.5%, respectively), but values did not differ between the 2 substrates.

Dog Diet Ingredient and Chemical Composition

Dietary ingredients included rice and corn as the carbohydrate sources, the test substrates as protein and lipid sources, poultry fat as an additional lipid source, and beet pulp as a dietary fiber source (Table 4). Diets were balanced with appropriate vitamins and minerals. Chemical composition was similar among diets. Crude protein concentrations were similar to the desired 30% value. Acid-hydrolyzed fat concentrations were close to the desired 20% value. Total dietary fiber, GE, and mineral concentrations likewise were similar among experimental diets.

Intake and Digestibility

No differences in nutrient intakes were noted among treatments (Table 5). Dogs ingested approximately 1.1% of BW daily, an ideal amount for adult dogs at maintenance. No differences in ileal nutrient digestibility were noted among treatments. Digestibility coefficients were increased for all nutrient categories, particularly AA and fat. Total tract nutrient digestibility data followed a similar pattern. No differences in fecal scores among diets were noted, with an average fecal score of 2.27 across treatments (data not shown). An average pH value of 6.5 was noted across treatments, with no statistical differences (data not shown).

Fecal Protein Catabolite Concentrations

Fecal ammonia concentrations were not different, averaging 45.8 μmol/g (DM basis) across treatments (data not shown). Biogenic amine concentrations (DM basis) were not different among treatments and had the following average concentrations: tryptamine (0.42 μmol/g), phenylethylamine (0.04 μmol/g), putrescine (1.06 μmol/g), cadaverine (0.40 μmol/g), histamine (0.05 μmol/g), tyramine (0.15 μmol/g), spermidine (1.64 μmol/g), and spermine (0.20 μmol/g). Fecal indole concentrations (DM basis) were greatest ($P < 0.05$) for dogs consuming the pollock diet (1.95

Table 3. Standardized digestibility (%) of AA in meat and fish substrates determined using the precision-fed cecectomized rooster assay[1]

	Substrate					
AA	Beef loin	Pork loin	Chicken breast	Pollock fillet	Salmon fillet	SEM
Essential						
Histidine	83.1^{bc}	81.0^{c}	75.7^{d}	87.4^{a}	84.5^{b}	0.92
Isoleucine	92.3^{a}	92.4^{a}	89.8^{b}	92.4^{a}	89.7^{b}	0.21
Leucine	92.4^{a}	92.3^{a}	89.9^{b}	92.6^{a}	89.8^{b}	0.25
Lysine	80.1	81.1	81.8	83.2	81.5	2.01
Methionine	93.3^{b}	93.4^{b}	90.8^{c}	93.9^{a}	90.8^{c}	0.01
Phenylalanine	91.5^{a}	91.5^{a}	89.4^{b}	91.6^{a}	89.1^{b}	0.28
Threonine	90.2^{b}	90.0^{b}	88.4^{c}	91.5^{a}	88.5^{c}	0.39
Tryptophan	90.8^{abc}	92.4^{ab}	89.1^{c}	92.9^{a}	90.2^{bc}	0.73
Valine	91.3^{a}	91.3^{a}	89.1^{b}	91.6^{a}	89.2^{b}	0.30
Nonessential						
Alanine	91.5^{a}	91.9^{a}	89.6^{b}	92.2^{a}	89.5^{b}	0.24
Arginine	79.1^{b}	77.5^{b}	77.3^{b}	87.2^{a}	79.6^{b}	1.73
Aspartic acid	91.3^{a}	91.2^{a}	88.9^{b}	90.9^{a}	88.8^{b}	0.21
Cysteine	83.3	82.7	82.0	84.7	81.9	1.01
Glutamic acid	90.1^{a}	89.9^{a}	87.2^{b}	91.4^{a}	87.4^{b}	0.56
Proline	88.3^{abc}	88.9^{a}	86.9^{c}	88.7^{ab}	87.2^{bc}	0.56
Serine	89.6^{b}	89.7^{b}	88.2^{c}	91.0^{a}	87.7^{c}	0.41
Tyrosine	91.6^{a}	91.6^{a}	87.0^{c}	92.1^{a}	89.1^{b}	0.33
TEAA[2]	89.0^{b}	89.0^{b}	86.6^{c}	90.4^{a}	87.8^{bc}	0.47
TNEAA[3]	88.1^{b}	87.9^{b}	85.9^{c}	89.8^{a}	86.4^{c}	0.49
TAA[4]	89.1^{b}	89.1^{b}	86.9^{c}	90.4^{a}	87.5^{c}	0.40

$^{a-d}$Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]Data are means of 4 roosters.
[2]TEAA = total essential AA.
[3]TNEAA = total nonessential AA.
[4]TAA = total AA.

μmol/g) compared with dogs consuming the pork loin (1.05 μmol/g) and chicken breast diets (0.96 μmol/g; data not shown). Dogs consuming the beef loin (1.19 μmol/g) and salmon fillet diets (1.53 μmol/g) had intermediate fecal indole concentrations.

DISCUSSION

The mammalian, avian, and marine substrates evaluated in this study were very good-quality cuts. During sample preparation, maintaining nutrient content and quality was emphasized. To achieve this, preservatives or additives, such as salt or antioxidants, were not used so as not to alter nutrient composition. Thaw times, dry times, and dryer load sizes were consistent for every test substrate in an attempt to preserve the quality of all substrates. In addition, a dehumidifying attachment was used on the dryer to allow for decreased drying temperatures (average 71°C). During substrate grinding, the bowl grinder prevented protein denaturation by allowing heat to dissipate easily because of bowl configuration.

Dry matter percentage values indicated that substrates were dried to a consistent moisture content. Ash content was consistent among mammalian and avian substrates. However, fish substrates had greater ash, Ca, P, and Mg concentrations, perhaps because of the presence of small bones embedded within the flesh. Pollock fillet, salmon fillet, and chicken breast CP concentrations were similar to values reported previously by Suvanich et al. (1998), Bechtel (2003), Lonergan et al. (2003), and Husak et al. (2008). Beef loin and pork loin CP concentrations were greater (2 to 16 and 4 to14 percentage units, respectively) compared with similar substrates studied by Happich et al. (1975), Novakofski et al. (1989), and Browning et al. (1990), likely because of the reduced fat concentration in the meat cuts. Among substrates, individual AA concentrations were generally similar. However, pollock fillet lysine concentration was 26% greater than those for beef loin and pork loin. Pollock fillet TEAA and TNEAA concentrations were greater than those for pork loin by 15 and 22%, respectively. Total AA concentrations were less than CP concentrations, indicating the presence of NPN components, such as biogenic amines, ammonia, and nucleotides.

Acid-hydrolyzed fat concentration was greatest for beef loin and pork loin, undoubtedly because of greater intramuscular fat compared with the other substrates. Polyunsaturated fatty acid and n-3 fatty acid concentrations were greatest for pollock and salmon fillets as a result of the increased n-3 fatty acid diet ingested by ocean fish.

Total dietary fiber in meat and fish cuts represents the presence of animal fiber (connective tissue; Otten et al., 2006). Values were small, indicating that sub-

Table 4. Ingredient and analyzed chemical composition of diets containing meat and fish substrates fed to ileally cannulated dogs

	Diet				
Item	Beef loin	Pork loin	Chicken breast	Pollock fillet	Salmon fillet
Ingredient, g/kg (as is)					
Test protein source	313.3	300.8	288.1	267.1	280.3
Poultry fat	148.4	154.8	166.7	183.5	172.0
Brewers rice	327.1	332.7	334.0	337.7	336.0
Ground corn	100.0	100.0	100.0	100.0	100.0
Beet pulp	70.0	70.0	70.0	70.0	70.0
Dicalcium phosphate	10.0	10.0	10.0	10.0	10.0
Choline chloride	3.0	3.0	3.0	3.0	3.0
Calcium carbonate	10.0	10.0	10.0	10.0	10.0
Vitamin premix[1]	2.0	2.0	2.0	2.0	2.0
Mineral premix[2]	2.0	2.0	2.0	2.0	2.0
Chromic oxide	2.0	2.0	2.0	2.0	2.0
Salt	6.5	7.0	7.0	7.0	7.0
Magnesium oxide	1.0	1.0	1.0	1.0	1.0
Potassium chloride	5.6	5.6	5.6	5.6	5.6
Chemical composition					
DM, %	94.3	94.2	93.9	93.8	94.0
OM, % (DM basis)	94.2	94.2	94.5	93.7	94.1
CP, % (DM basis)	30.7	31.2	30.0	32.0	30.8
Acid-hydrolyzed fat, % (DM basis)	21.1	21.4	21.4	20.1	20.4
Total dietary fiber, % (DM basis)	5.3	4.7	5.5	4.9	4.9
GE, kcal/g	5.4	5.4	5.4	5.3	5.3
Mineral, % (DM basis)					
Ca	0.70	0.71	0.74	0.80	0.76
P	0.52	0.54	0.55	0.57	0.58
Fe	0.05	0.05	0.04	0.06	0.06
Mg	0.13	0.14	0.14	0.14	0.15
Zn	0.04	0.04	0.04	0.04	0.04

[1]Provided per kilogram of diet: vitamin A, 5.28 mg; vitamin D_3, 0.04 mg; vitamin E, 120 mg; vitamin K, 0.88 mg; thiamine, 4.40 mg; riboflavin, 5.72 mg; pantothenic acid, 22.00 mg; niacin, 39.60 mg; pyridoxine, 3.52 mg; biotin, 0.13 mg; folic acid, 0.44 mg; vitamin B_{12}, 0.11 mg.

[2]Provided per kilogram of diet: Mn (as $MnSO_4$), 66.00 mg; Fe (as $FeSO_4$), 120 mg; Cu (as $CuSO_4$), 18 mg; Co (as $CoSO_4$), 1.20 mg; Zn (as $ZnSO_4$), 240 mg; I (as KI), 1.8 mg; Se (as Na_2SeO_3), 0.24 mg.

Table 5. Nutrient intakes and digestibility values of diets containing meat and fish substrates by ileally cannulated dogs (n = 5)

	Diet					
Item	Beef loin	Pork loin	Chicken breast	Pollock fillet	Salmon fillet	SEM
Intake, g/d						
DM	277.8	272.2	249.6	265.4	246.1	20.8
OM	261.6	256.4	235.8	248.7	231.4	19.6
CP	85.3	84.8	75.0	84.9	75.9	6.4
Acid-hydrolyzed fat	58.5	58.2	53.4	53.5	50.1	4.3
Digestion at ileum, %						
DM	86.7	86.6	86.1	87.4	86.5	0.9
OM	89.5	89.4	89.0	89.9	89.2	0.8
CP	89.7	90.5	88.9	90.5	89.2	1.3
TEAA[1]	92.6	92.7	91.7	92.3	91.8	1.1
TNEAA[2]	90.3	90.4	88.8	89.4	89.6	1.3
TAA[3]	91.5	91.6	90.4	91.0	90.7	1.2
Acid-hydrolyzed fat	97.3	97.4	97.0	97.7	97.6	0.5
Total tract digestion, %						
DM	92.4	92.3	92.6	92.5	92.7	0.1
OM	94.5	94.5	94.7	94.6	94.7	0.1
CP	94.4	94.7	94.7	94.8	94.7	0.2
Acid-hydrolyzed fat	97.4	97.5	97.4	97.5	97.5	0.1

[1]TEAA = total essential AA.

[2]TNEAA = total nonessential AA.

[3]TAA = total AA.

strates were of good quality. However, collagen concentrations in our protein substrates were greater compared with those reported by researchers evaluating beef loin (2.0%), pork loin (1.9%), and chicken breast (1.7%; McKeith et al., 1985; DeVol et al., 1988; Lan et al., 1995). Our fish substrates had smaller collagen concentrations than the carp fillet and salmon fillet (1.8 and 2.1%, respectively) evaluated by Sato et al. (1986). Differences in collagen concentration could be due to the assay used to measure hydroxyproline. In our study, hydroxyproline was measured by HPLC, whereas other studies (McKeith et al., 1985; DeVol et al., 1988; Lan et al., 1995) used a spectrophotometric absorption assay (Blumenkrantz and Asboe-Hansen, 1975), which could underestimate hydroxyproline concentrations and lead to smaller calculated collagen concentrations. Sato et al. (1986) used a similar chemical extraction method involving spectrophotometric absorption, which also could have resulted in different hydroxyproline concentrations.

Biogenic amine concentration data are used to evaluate the freshness and safety of meat and fish. For example, putrescine concentrations increase as meat and fish begin to decay (Mietz and Karmas, 1978). In the current study, the putrescine concentration in beef loin was similar to the beef loin concentration reported by Eliassen et al. (2002), but all other test substrate putrescine concentrations were 10 times greater than for similar protein substrates evaluated in their study. Cadaverine concentrations in all test substrates were similar to the data of Eliassen et al. (2002) and Silva and Glória (2002), who reported concentration values on an as-is basis, with no moisture percentages reported. In addition, they evaluated fresh meat and fish cuts, whereas our concentrations are reported on a DM basis and tests were conducted on dried cuts of meat and fish. These factors could explain differences in biogenic amine concentrations among studies.

Spermidine concentrations of our protein substrates were similar compared with those from other studies evaluating similar protein substrates (Silva and Glória, 2002; Kalač, 2006). Beef loin, pork loin, and chicken breast spermine concentrations (0.25, 0.69, 0.92 μmol/g, respectively; DM basis) were greater in our study compared with those from other studies evaluating spermine concentrations (as-is basis) in similar substrates (0.03 to 0.14, 0.07 to 0.17, 0.09 to 0.40 μmol/g, respectively). In these studies, moisture percentages were not reported; therefore, an exact comparison is not possible. Histamine and tyramine concentrations in beef, pork, and chicken were not different from other reported values (Silva and Glória, 2002; Kalač, 2006).

Beef loin contained twice the Fe concentration compared with other substrates. Beef loin is a red meat containing elevated myoglobin concentrations. Pork, also a red meat, contains smaller myoglobin concentrations than beef, hence the decreased Fe concentration. These results are in agreement with data reported by Leonhardt and Wenk (1997) and Zarkadas et al. (1987). Zinc concentrations were greatest in beef loin and pork loin compared with chicken breast, salmon fillet, and pollock fillet, which were similar in Zn concentration. These data are similar to those of Zarkadas et al. (1987), Leonhardt and Wenk (1997), and Sheeska and Murkin (2002). Mercury was not detected in any of the protein substrates. This was an initial concern in the case of ocean fish, in which increased mercury concentrations can sometimes be found. This concern proved to be unwarranted with the ingredient sources used in the current study.

The Poultry Complete IDEA kit was designed to analyze a variety of animal protein sources, such as meat and bone meals, poultry by-product meals, and meat meals, rather than good-quality meat and fish cuts. This factor could have affected our results because of the use of calculations based on standardized AA digestibility of animal by-products by roosters. Predicted digestibility values of individual AA are based on the IDEA value, which explains why digestibility rankings among substrates were consistent. Pollock fillet AA were predicted to have very high digestibility values, indicating that the peptide bonds were easily hydrolyzed by the enzymes used in the assay. Peptide bonds in chicken breast apparently were not as easily hydrolyzed, leading to smaller predicted digestibility values. This is well correlated with cecectomized rooster data, in which pollock fillet had the greatest AA digestibility values and chicken breast the smallest.

On average, the IDEA assay predicted individual AA digestibility values that were approximately 6 and 4 percentage units greater for pollock fillet and salmon fillet, respectively, than was observed in the cecectomized rooster assay. In contrast, chicken breast was predicted to be 3 percentage units less than was observed in the cecectomized rooster assay.

The IDEA assay often is used to predict lysine digestibility in protein sources. In our study, this assay predicted lysine digestibility to be approximately 10 percentage units greater than was observed in the cecectomized rooster assay for all substrates except chicken breast, which was 1 percentage unit greater. This difference could be due to the inability of the kit to analyze a protein substrate that was above the quality standard for which it was designed. Overall digestibility rankings among substrates were similar between the IDEA and cecectomized rooster assays.

Only 2 published studies (Dust et al., 2005; Folador et al., 2006) have reported IDEA values for meat and fish substrates. Dust et al. (2005) reported a value of 0.64 for spray-dried chicken, which was 0.12 units greater than our value for chicken breast. Folador et al. (2006) reported a value of 0.71 for white fish meal, which is the same value that we calculated for pollock fillet.

The cecectomized rooster results indicate that standardized AA digestibility was elevated for all substrates. Pollock fillet AA digestibility values were either equal to or greater than those for other substrates, indicating

that peptide bonds may be more easily hydrolyzed, allowing AA to be absorbed by the rooster. Beef loin and pork loin AA digestibility values were similar. Chicken breast and salmon fillet also did not differ in AA digestibility except for tyrosine, with salmon fillet being greater than chicken breast. No lysine or cysteine digestibility differences were detected among substrates. Red meat (beef loin and pork loin) sources were similar in AA digestibility, whereas fish sources differed.

Amino acid digestibility can be affected by factors such as the presence of connective tissue, ash, and the processing temperature used to prepare the protein source. These factors were minimized in our study, allowing the dietary nutrients to be readily digested, and presumably absorbed and utilized, by the roosters. Although beef loin and pork loin, compared with the other test substrates, had greater total dietary fiber and collagen concentrations, indicating the presence of connective tissue, this amount of connective tissue did not negatively affect digestibility. Indeed, greater AA digestibility values for beef loin and pork loin were noted compared with chicken breast and salmon fillet. A similar response was noted for pollock fillet, which had a greater ash concentration than the other substrates, yet tended to have the greatest AA digestibility.

The diet used in the ileally cannulated dog assay was of very good quality. The test protein substrate was the only ingredient that differed among diets, thus equalizing the effects of other nutrients on diet digestibility. All diets were similar in composition and were similar to the formulated composition of 30% CP and 20% fat. These diet similarities allowed differences in the test substrate to be the factor affecting nutrient digestibility, as opposed to differences in ingredient and nutrient compositions of the diets. In addition, food intakes were statistically similar, allowing for a more precise measure of nutrient digestibility.

Apparent ileal and total tract digestibility values of CP were large for all diets. Much smaller apparent ileal digestibility of CP (28 to 34 percentage units) and smaller apparent total tract digestibility of CP (5 to 14 percentage units) are noted when ileally cannulated dogs or pigs are fed protein by-products [e.g., fish meal, poultry by-product meal, or meat meal diets (Jørgensen et al., 1984; Knabe et al., 1989; Zuo et al., 1996; Murray et al., 1997; Johnson et al., 1998; Bednar et al., 2000; Yamka et al., 2003)]. This undoubtedly is due to the protein quality of the test substrates. Factors such as the presence of connective tissue, ash content, and the processing temperature used to prepare the protein source decrease digestibility (Kies, 1981; Friedman, 1996; Parsons, 2002). These factors were minimized in our study, allowing the dietary nutrients to be readily digested, and presumably absorbed and utilized, by the dogs. Although beef loin, pork loin, and chicken breast, compared with the other substrates, had greater total dietary fiber and collagen concentrations, indicating the presence of connective tissue, this concentration of connective tissue and the 30% inclusion rate of beef, pork, and chicken into the diet limited potential negative responses in digestibility.

Digestibility differences between the ileum and feces represent the contribution of the large bowel to total tract nutrient digestibility. Dry matter digestibility was approximately 6 percentage units greater in the total tract compared with the ileum. Organic matter and CP total tract digestibility increased by approximately 5 percentage units between the ileum and feces. Acid-hydrolyzed fat did not change because of the inability of anaerobic bacteria to digest fat, owing to the reductive environment of the large bowel. These differences in digestibility indicate that nutrients were digested primarily by gastric and small intestinal (i.e., hydrolytic) processes.

Few differences were noted among treatments in fecal pH and protein catabolite (indole, phenol, and biogenic amine) concentrations, another indication that most nutrients, especially protein and AA, were digested extensively anterior to the large bowel. Indeed, of all the phenolic and indolic compounds analyzed, only indole and 2,3-dimethyl indole were detected in feces. Much greater concentrations of these compounds, and greater numbers of phenolic and indolic compounds, are detected when lesser quality proteins are fed (Swanson et al., 2002a,b; Flickinger et al., 2003).

In summary, the good-quality protein substrates (beef loin, pork loin, chicken breast, pollock fillet, and salmon fillet) tested in this experiment have major differences in chemical composition but relatively minor digestibility differences. Among these sources, pollock fillet was shown to be the most digestible protein and chicken breast the least, based on predicted and standardized AA digestibility values. Beef loin and pork loin were similar in digestibility. Salmon fillet was similar in digestibility compared with beef loin and pork loin based on IDEA; however, smaller standardized AA digestibility values were noted. When the protein sources were incorporated into a diet supplying 25% of total energy intake, no significant differences existed in either ileal or total tract apparent nutrient digestibility when using the ileally cannulated dog model. Results demonstrate that good-quality proteins from mammalian, avian, and marine sources are hydrolytically digested in an efficient manner, leaving little residual material to be digested by the anaerobic microbiota in the large bowel. This is not the case when proteinaceous ingredients of lesser nutritive value are fed.

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